DNA POLYMORPHISMS IN DENTAL PULP: EFFECT OF ENVIRONMENTAL FACTORS.

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This study was designed to observe the results of DNA typing on teeth subjected to aging, different temperatures and various environmental factors. The study includes the analysis of some DNA polymorphisms amplified by PCR, such as the HLA DQA1, D1S80, two STRs (HUMTH01 and HUMFES/FPS) and sex typing (XY homologous gene amelogenin).

Material and methods

Samples

Teeth were obtained from patients of oral surgeons (n=559), rinsed with distilled water, air dried and inmediately frozen at -20°C after the extraction until teeth were exposed to the experimental conditions.

Conditions of exposure (series)

1. Temperature
198 teeth were removed from the freezer and mantained at 4°C, 20°C and 40°C for periods of time ranging from 15 days (2weeks) to 36 months.
2. Water
96 teeth were submerged in the sea and in a river for periods of time ranging from 15 days to 6 months.
3. Burial
72 teeth were buried outdoors in a garden and in the sand of a beach (Pontevedra). They were placed approximately 20 cms into the ground for periods of time ranging from 15 days (2 weeks) to 6 months.
4. Outdoors air exposure
36 teeth were exposed to open air for periods of time ranging from 15 days (2 weeks) to 6 months.
5. Incineration
We incinerated 144 teeth by introducing them in a dental ceramic furnace (Programat P95, Ivoclar) for 1 and 2 min at 75°C, 100°C, 200°C, 300°C, 400°C and 500°C.
6. Aging (old samples)
Six teeth of 10 to 30 years old were mantained at indoor conditions.
7. Forensic casework
Three teeth from cremated bodies (corpses) and one from an exhumated body (of more than 50 years buried) from our forensic casework were included in our study.

Samples preparation and DNA extraction

After removing teeth from experimental conditions of exposure, they were rinsed with distilled water and air dried. The access to dental pulp was performed using three different methods (Smith et al 1993): mostly of the teeth were enterely crushed and the others were either opened by a conventional endodontic access or cut with a transversal section. Dental pulp was retrieved by a fine forceps and resuspended in water for DNA extraction with chelating resine (Singer-Sam 1989).
DNA was quantified using the DNA DipStick kit (Invitrogen Corp.)

Detection methods

System	Method
HLA DQA1	Dot-Blot with ASO probes
D1S80	PAGE+ Silver Staining
HUMTH01	PAGE+ Silver Staining
HUMFES/FPS	PAGE+ Silver Staining
XY Homologous gene amelogenin	ALF DNA Sequencer

Results

Table1. Results of HLA DQA1 system under different conditions of exposure and time periods.

Time	Conditions of exposure							
	4°C	20°C	40°C	fresh water	seawater	outdoors	sand buried	soil buried
15 days	6/6	6/6	6/6	3/6	6/6	6/6	6/6	5/6
1 month	5/6	6/6	6/6	1/6	1/6	6/6	4//6	5/6
3 months	5/6	6/6	6/6	1/6	0/6	4/6	3/6	4/6
6 months	4/6	5/6	5/6	1/6	1/6	4/6	2/6	2/6
12 months	6/6	5/6	5/6					
24 months	6/6	6/6	6/6					
36 months	5/6	6/6	6/6					

positive results/sample number

Table 2. Results of D1S80 system under different conditions of exposure and time periods.

Time	Conditions of exposure							
	4°C	20°C	40°C	fresh water	seawater	outdoors	sand buried	soil buried
3 months	6/6	6/6	6/6	2/6	2/6			
6 months				1/6	1/6	3/6	2/6	3/6
12 months	6/6	5/6	4/6					
36 months	5/6	4/6	3/6					

positive results/sample number

Table 3. Results of HUMTH01 locus under different conditions of exposure and time periods.

Time	Conditions of exposure							
	4°C	20°C	40°C	fresh water	seawater	outdoors	sand buried	soil buried
3 months				4/6	4/6			
6 months				2/6	2/6	5/6	4/6	3/6
36 months	6/6	6/6	6/6					

positive results/sample number

Table 4. Results of HUMFES/FPS locus under different conditions of exposure and time periods.

Time	Conditions of exposure							
	4°C	20°C	40°C	fresh water	seawater	outdoors	sand buried	soil buried
3 months				4/6	6/6			
6 months				3/6	2/6	5/6	5/6	3/6
36 months	6/6	6/6	6/6					

positive results/sample number

Table 5. Results of XY homologous gene amelogenin under different conditions of exposure and time periods.

Time	Conditions of exposure							
	4°C	20°C	40°C	fresh water	seawater	outdoors	sand buried	soil buried
3 months				6/6	6/6			
6 months				6/6	6/6	6/6	6/6	6/6
36 months	6/6	6/6	6/6					

positive results/sample number

Table 6. Results of the PCR markers analized after the teeth cremation with the temperatures and time used.

	HLA DQA1		D1S80	HUMTH01	HUMFES/FPS	XY Amelogenin
	1 min	2 min	2 min	2 min	2 min	2 min
75°C	6/6	6/6	6/6	6/6	6/6	6/6
100°C	6/6	5/6	6/6	6/6	6/6	6/6
200°C	6/6	6/6	5/6	6/6	6/6	6/6
300°C	6/6	5/6	1/6	6/6	5/6	6/6
400°C	6/6	2/6	0/6	4/6	4/6	6/6
500°C	2/6	0/6	0/6	0/6	0/6	5/6

positive results/sample number

Discussion

1. Temperature and aging

During the testing period of 36 months using temperatures of 4, 20 and 40°C positive results were obtained in most of the cases.

100% positive results were obtained for the two STRs studied and the amelogenin gene. Good results were also obtained for HLA DQA1 and D1S80 (more than 50% in the oldest samples).

There were no significant differences between the three temperatures studied (4, 20,40°C) and the results were similar for all three.

2. Water

Near 50% positive results were obtained typing STRs in samples submerged in water. Worst results (1/6) were obtained for HLA DQA1 and D1S80 and the best ones (6/6) for the XY homologous gene amelogenin. No significant differences were obtained between seawater and fresh water.

In general teeth submerged in water offer the poorest results. It is neccesary to keep in mind that the average temperatures of seawater and fresh water in Galicia range from 10-20°C and both are extremely rich in zooplankton. This explains the bad results obtained for this serie.

3. Outdoors/buried

Teeth exposed outdoors offer better results than burial teeth, but even in this case an average of 50% positive results were obtained in the 6 month old samples. Slightly better results were obtained when using STRs.

No significant differences were seen between teeth buried in sand or soil.

4. Incinerated teeth

With the exception of the amelogenin gene, complete negative results were obtained after exposure of 500°C during 2 min. The STRs offer clearly better results than HLA DQA1. The latter offers better results than D1S80 (only 1/6 at 300°C).

If the exposure time to high temperatures is reduced (1min), clearly better results are obtained.

5. Old samples

Teeth ranging from 10 to 30 years old were studied. 100% positive results were obtained for all the systems with the exception of D1S80 where only 50% positive results were observed.

6. Markers and aging

In general the best results were obtained with the amelogenin gene followed by the two STRs studied (HUMTH01 and HUMFES/FPS). The small sizes and the method of detection used after PCR amplification are the main factors in explaining this fact.

References

Singer-Sam, J., Tanguay, R.L., Riggs, A.D. (1989). "Use of chelex to improve the PCR signal from a small number of cells". *Amplications: A forum for PCR Users.*

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